Detection of Cox-1 in Formalin-Fixed, Paraffin-Embedded in Mouse Tissue

Reagents:

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information:

Kit used: Vector M.O.M. Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog: PK2200

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Primary antibody: Mouse anti-sheep Cox 1
Caymen Chemical
Ann Arbor, MI 48108
www.caymanchem.com
1-800-364-9897
Catalog #160110

Negative Control: Normal Mouse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #015-000-001

Staining Procedure:

-Positive Control Tissue: Mouse Male Reproduction, vas deferens, epididymis
-Stain Localization: Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Perform Heat Induced Epitope Retrieval using Microwave Oven.
Place a full rack of slides in a container containing 200 mls 1X citrate buffer.
MWO for 5 minutes at power level 3
Cool for 1 minute (Add 50 mls citrate buffer to container)
MWO for 5 minutes at power level 3 temp_____
Cool 20 minutes at room temperature
Rinse in distilled water 3 X 2 minutes each
Place slides in buffer for 5 minutes

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Incubate sections for 1 HOUR in MOM specific IgG blocking reagent.
(Made via 2.5 mls 1x PBS plus 2 drops of Mouse IgG blocking reagent)
Lot#_____ Exp Date_____

6. Apply Avidin/Biotin block
Lot#_____ Exp Date_____ New Kit yes / no
Apply avidin block - 15 min @ RT.
Quick rinse in 1X AB.
Apply biotin block - 15 min @ RT.
No wash, wipe excess block and apply primary antibody
Wipe excess reagent from around tissue section.

DO NOT RINSE SECTIONS WITH BUFFER.

Make primary antibody dilution and secondary in Vector MOM diluent. (600ul of protein stock in 7.5 mls PBS)

7. Apply primary antibody at a 1:25 dilution and incubate for one hour.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody and use this to make 1:25 dilution. Apply to slides and Incubate for one hour.
Lot#_____ reconstituted date_____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply M.O.M. biotinylated anti-mouse IgG and incubate for 10 minutes
Made via 10ul of antibody in 2.5mls of Vector MOM diluent.

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Vectastain ABC Elite label for 5 minutes.
(Made via 2 drops of Reagent A plus 2 drops of Reagent B in 2.5 mls BSA diluent. Prepare 30 minutes before use)
Exp Date_____ New Kit Yes / No

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot #_____ Exp Date_____ New Kit Yes / No

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

17.Rinse slides in 1x automation buffer for 1 min with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip
updated 01/14/2004